METHODS AND MATERIALS FOR OPTIMIZATION OF ELECTRONIC HYBRIDIZATION REACTIONS

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The following inventions relate to discoveries concerning the various parameters, electrolytes (buffers), and other conditions which improve or optimize the speed of DNA transport, the efficiency of DNA hybridization reactions, and the overall hybridization specificity in microelectronic chips and devices. In particular, this invention relates to the discovery that low conductance zwitterionic buffer solutions, especially those containing the amino acid Histidine prepared at concentrations of SIMILAR 50 mM and at or near the pI (isoelectric point SIMILAR pH 7.47), provide optimal conditions for both rapid electrophoretic DNA transport and efficient hybridization reactions. Hybridization efficiencies of at least a factor of 10 relative to the next best known buffer, Cysteine, are achieved. Test data demonstrate an approximately 50,000 fold increase in hybridization efficiency compared to Cysteine.

CLAIMS

[Claim(s)]
1.(1) Apply the low conductivity buffer solution to equipment. (2) equipment is made to produce electric field by the trial part with the application of a current. A target-nucleus acid is conveyed to (3) trial part. Subsequently About the cysteine under (4) same conditions, at more at least 10 times larger hybridization effectiveness How to make a target-nucleus acid convey and hybridize in the micro electronic instrument which has at least one trial part which bears the capture nucleic acid characterized by including the process which hybridizes a target-nucleus acid to a capture nucleic acid by the trial part.
2. Approach according to claim 1 the low conductivity buffer solution is the zwitter-ion buffer solution.
3. Approach according to claim 2 the zwitter-ion buffer solution contains histidine.
4. Approach according to claim 3 by which histidine was prepared by concentration of about ten to 100 mM.
5. A histidine is the isoelectric point or the approach according to claim 3 about prepared in the isoelectric point.
6. Approach according to claim 1 the isoelectric point is abbreviation pH 7.47.
7. Approach according to claim 1 buffer nature existence object stabilizes hybridization between target-nucleus acid and capture nucleic acid.
8. Approach according to claim 7 buffer nature existence object is natural compound which has low conductivity.
9. Approach according to claim 7 buffer nature existence object is natural zwitter-ion compound.
10. The approach according to claim 7 a buffer nature existence object is a synthetic compound which has low conductivity.
11. The approach according to claim 7 a buffer nature existence object is a composite zwitter-ion compound.
12. Hybridization effectiveness is a more at least 100 times larger approach according to claim 1 about the cysteine under the same conditions.
13. Hybridization effectiveness is a more at least 1,000 times larger approach according to claim 1 about the cysteine under the same conditions.
14. Hybridization effectiveness is an about 50,000 times larger approach [at least] according to claim 1 about the cysteine under the same conditions.
15. The approach according to claim 1 a buffer nature existence object reduces the repulsive force between a capture nucleic acid and a target-nucleus acid.
16. The approach according to claim 1 the buffer solution reduces the addition product formation between a capture nucleic acid and a target-nucleus acid.
17. Apply Low Conductivity Buffer Solution to Equipment. Power is applied to equipment. By the minute location trial part of equipment The electrical and electric equipment of a target-nucleus acid Migration transportation and are recording are caused. Subsequently About the cysteine under the same conditions, at more at least 10 times larger effectiveness How to reinforce electrophoresis transportation of a target-nucleus acid and hybridization effectiveness in micro electronic hybridization including the minute location trial part which is characterized by including the process which makes a target-nucleus acid hybridize and which has a capture nucleic acid.
18. The approach according to claim 17 the low conductivity buffer solution is the zwitter-ion buffer

solution.
19. The approach according to claim 18 the zwitter-ion buffer solution contains a histidine.
20. The approach according to claim 19 by which the histidine was prepared by the concentration of about ten to 100 mM.
21. A histidine is the isoelectric point or the approach according to claim 19 about prepared in the isoelectric point.
22. Approach according to claim 17 the isoelectric point is abbreviation pH 7.47.
23. The approach according to claim 17 a buffer nature existence object stabilizes the hybridization between a target-nucleus acid and a capture nucleic acid.
24. The approach according to claim 23 a buffer nature existence object is a natural compound which has low conductivity.
25. The approach according to claim 23 a buffer nature existence object is a natural zwitter-ion compound.
26. The approach according to claim 23 a buffer nature existence object is a synthetic compound which has low conductivity.
27. The approach according to claim 23 a buffer nature existence object is a composite zwitter-ion compound.
28. Hybridization effectiveness is a more at least 100 times larger approach according to claim 17 about the cysteine under the same conditions.
29. Hybridization effectiveness is a more at least 1,000 times larger approach according to claim 17 about the cysteine under the same conditions.
30. Hybridization effectiveness is an about 50,000 times larger approach [at least] according to claim 17 about the cysteine under the same conditions.
31. The approach according to claim 17 a buffer nature existence object reduces the repulsive force between a capture nucleic acid and a target-nucleus acid.
32. The approach according to claim 17 the buffer solution reduces addition product formation of a capture nucleic acid and a target-nucleus acid.

[Translation done.]

* NOTICES *

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1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
Electro nick hybridization reaction The approach for optimization, and field of matter invention This invention relates to the buffer solution and the electrolytic solution which are used for the electronic instrument which suits a medicine diagnostic application, a biological application, and other applications. It is related more with the buffer solution and the electrolytic solution in the DNA-hybridization analysis carried out to a detail by micro electronic medicine diagnostic equipment aiming at advantageous use.
Background of invention The interest about the equipment which combined micro electronics and molecular biology is increasing in recent years. It applies on November 1, 1993, and one of the systems of these is indicated by "ACTIVE PROGRAMMABLE ELECTRONIC DESVICE FOR MOLECULAR BIOLOGICAL ANALYSIS AND DIAGNOSTICS" of the serial number 08th exhibited as current U.S. Pat. No. 5,605,662 / No. 146,504, it carries out source designation here, and regards as some of these specifications. The system indicated there is called APEX system. In nucleic-acid hybridization, an antibody / antigen reaction, a clinical diagnosis, and the molecule biological reaction like biopolymer composition, an APEX system is used advantageously and can achieve an extensive function.
APEX mold equipment uses the buffer solution and the electrolytic solution for those actuation. The buffer solution is defined as chemical solution which has resistance in pH change to addition of an acid or alkali. For example, refer to Dictionary of Biotechnology, the 2nd edition, James Coombs, and stockton Press. As indicated there, it is "having used the buffer solution based on mineral salt (phosphate, carbonate) and organic salt (acetate, citrate, succinate, a glycine, a maleate, barbiturates, etc.) in the biology experiment traditionally."
It is the purpose of this invention to find out the buffer solution and the electrolytic solution which are advantageously used in the molecule biological electronic instrument which performs hybridization, reaction, diagnosis, and composition.
Outline of invention The following invention relates to the discovery relevant to the parameter, the various electrolytic solution (buffer solution), and other various conditions which improve or optimize the effectiveness and the total hybridization singularity of the DNA transportation rate in this invention persons' APEX micro electronic chip and equipment, and a DNA-hybridization reaction. This invention especially relates to discovery that the thing containing low conductivity zwitter-ion buffer solution and the amino acid histidine preferably prepared ten to 100 mM the concentration of about 50 mM(s), pI (about [isoelectric point] pH 7.47), or near the offers optimization conditions about quick DNA transportation and an efficient hybridization reaction at a detail. One at least 10 times the hybridization effectiveness of this is attained to the buffer solution with which the degree was known best, and a cysteine. A test data shows about 50,000 times as many increase as this in hybridization effectiveness as compared with a cysteine.
Easy diagrammatic publication Drawing 1 is the top view of the checker board array using the histidine buffer solution.
Detailed publication of invention In the electrolytic solution/buffer solution various type, there are various physical parameters related to electrophoresis transportation and other electric charge analyte of DNA. It is a certain kind of equipment, for example, DC (direct current) electric apparatus with which the APEX equipment of the applicant like a publication generates electric field fundamentally to aforementioned U.S. Pat. No. 5,605,662 in a device table side. Subsequently, these places cause electrophoresis transportation of

an electric charge molecule between the minute locations (microlocation) which inclined contrary to the device table side (+/-). By contrast, the so-called JINOSENSA (Genosensor) (impedance sensor) (For example, refer to WO93/22678 of Holles(es) et al. and "Optical and Electrical Methods and Apparatus for Molecular Detection") And two-dimensional-electrophoresis (dielectrophoresis) equipment (for example, Washizu 25 Journal of Electrostatics) 109-123 and 1990 reference include use in AC electric field. When, as for the material-difference point about these equipments, these AC places are impressed, also in any of these systems, there is essentially no net current, namely, the driving force of the electrophoresis for electric charge molecule transportation is the point which is not. APEX mold equipment produces the direct current (DC) of remarkable net, when an electrical potential difference is impressed, and it is recognized as "a sign of electrophoresis." In electrophoresis, migration of ion or a charged particle is produced according to the electric force which met in the direction of an electric field gradient, and the relation between a current and an electrical potential difference is important for this technique. This electrophoresis-migration is macroscopically shown in itself as current conductivity under the effect of the impressed electrical potential difference, and in a solution. V=RxI V is potential. R is [VxA-1=R (omega)] which is the electric resistance of the electrolytic solution.
I is a current [A].
** Ohm's law is followed.
Resistance of a solution is the inverse number of the conductivity which can be measured with conductometer. As for conductivity, depending on the ion kinds and those concentration of the buffer solution/electrolytic solution,;, therefore these parameters are mainly very important for an electric-field related molecule biological technique. The produced electric field are essentially really the same [fundamental current / electrical-potential-difference relation] in it being about which other electrophoresis systems per APEX technique similarly in a microscopic environment.
An APEX system has the unique description about how the various approaches of supplying a current and an electrical potential difference, the current, and the plot of an electrical potential difference were found out although the engine performance of this system is improved. Seemingly, some DC pulse technique (linearity and a logarithm inclination) will provide a detail with the improved hybridization stringency.
Electrophoresis transportation-counter ion reinforcement There is reduction for numerical in the mobility of electric charge analyte kinds (protein, DNA, etc.), and it may set in the field of electrophoresis that it is in inverse proportion to the square root of the ionic strength of an electrolyte, and it is established (83 pages and drawing 3 .16 reference of "Capillary Electrophoresis:Principles and Practice", R.Kuhn and S.Hoffstetter, Springer-Verlag, and 1993). With one of given fixed electric field strength, the analyte will be conveyed at the quicker rate as electrolytic-solution concentration decreases to analyte kinds (protein, DNA, etc.). The same result which shows this effectiveness about dansyl amino acid is J.J.Issaq et al. and Chromatographia. It is shown by 155 thru/or 161 pages (refer to 157-page drawing 3 in a detail) Vol.32, and eight #3 / months, 1991 [4 or]. The result which shows about DNA that this effectiveness is a different electrolyte is P.D.Ross and R.L.Scruggs, and Biopolymers. It is shown in Vol.2,231 thru/or 236 pages, and 1964 (refer to 232-page drawing 1 in a detail).
Relation between ionic strength/conductivity
溶液（Ｎａ$^{+}\longleftrightarrow$Ｃｌ$^{-}$、Ｋ$^{+}\longleftrightarrow$Ｃｌ$^{-}$等）中の完全解離したアニオンおよび

About the un-buffering nature electrolytic solutions (a sodium chloride, potassium chloride, etc.) containing a cation, ionic strength and conductivity are equivalent, namely, conductivity is usually proportional to ionic strength. であろう。それらの解離状態（例：２Ｎａ$^{+}\longleftrightarrow$ＰＯ$_{4}^{--2}$）にある緩衝性電解質

About (phosphate, acetate, citrate, succinate), etc., ionic strength and conductivity will usually become equivalence, namely, conductivity is proportional to ionic strength.
Conductivity will decrease about 10 times per the isoelectric point (pI) and (electric dissociation exponent) ** pH unit difference of a between about the buffer nature electrolytic solution [the good buffer solution (MOPS, HEPES, TAPS, fricin, vicine), the amino acid buffer solution, the both-sexes electrolytic solution], etc. which can have a zwitter-ion kind (a net electric charge is not carried out in those pI(s)). For example, for the amino acid in the zwitter-ion condition (-OOC-CH(R)-NH3+), an "amino acid part" is.

十分な正味の正電荷（$HOOC-CH(R)-NH_2^+ \longleftrightarrow X^-$）または十分な正味の負の電荷（$Y^+ \longleftrightarrow {}^-OOC-CH(R)-NH_2$）を有する場合よりおよそ１０

Probably, it has the 00 times lower conductivity value. Therefore, negative [formal] or positive charge occurs in an amino acid part, and, probably, conductivity and ionic strength begin to be related as it separates from the pI. However, pI or near the, probably, conductivity will be very low rather than it is expected by given ionic strength or concentration. When used pI or near [its] those, the text of electrophoresis will make reference, if the good buffer solution and the amino acid buffer solution have "it is low conductivity at high ionic strength or concentration" (refer to Capillary Electrophoresis:Principles and Practice", R.Kuhn and S.Hoffstetter, Springer-Verlag, and 88 pages of 1993). Usually, in practice, the electrophoresis buffer solution "a tris-boric acid" used has quite low conductivity rather than it is expected from the ionic strength or concentration. ********** [this / for the "tris cation" which forms the zwitter-ion complex comparatively stabilized in the solution, and a "boric-acid anion"]. The conductivity of a 100mM tris-boric-acid solution was measured as it is 694microS/cm, and this was about 20 times lower rather than it was expected from the ionic strength, and it was equivalent to 5mM sodium phosphate or a sodium chloride solution about. Table 1 shows the conductivity measured value of much transportation buffer solutions.

溶液/緩衝液	測定 1	測定 2	測定 3	平均/標準偏差
10 mM $MgCl_2$	1.95 mS/cm	2.02 mS/cm	2.13 mS/cm	2.03+/-0.09 mS/cm
1 mM $MgCl_2$	174 μS/cm	208 μS/cm	177 μS/cm	186+/-18.8 μS/cm
0.1 mM $MgCl_2$	16.9 μS/cm	16.7 μS/cm	18.3 μS/cm	17.3+/-0.87 μS/cm
10 mM NaCl	1.07 mS/cm	1.10 mS/cm	1.18 mS/cm	1.12+/-0.057 mS/cm
1 mM NaCl	112 μS/cm	115 μS/cm	111 μS/cm	112.7+/-2.08 μS/cm
0.1 mM NaCl	8.80 μS/cm	8.98 μS/cm	10.5 μS/cm	9.43+/-0.93 μS/cm
20 mM $NaPO_4$	2.90 mS/cm	2.79 mS/cm	3.00 mS/cm	2.90+/-0.11 mS/cm
10 mM $NaPO_4$	1.40 mS/cm	1.44 mS/cm	1.48 mS/cm	1.44+/-0.04 mS/cm
1 mM $NaPO_4$	122 μS/cm	128 μS/cm	136 μS/cm	128.7+/-7.0 μS/cm
50 mM TRIS	3.50 mS/cm	3.14 mS/cm	3.40 mS/cm	3.35+/-0.19 mS/cm
10 mM TRIS	572 μS/cm	562 μS/cm	583 μS/cm	572+/-10.5 μS/cm
250 mM HEPES	141 μS/cm	144 μS/cm	158 μS/cm	147.6+/-9.07 μS/cm
25 mM HEPES	9.16 μS/cm	9.44 μS/cm	10.5 μS/cm	9.7+/-0.71 μS/cm
3.3 mM クエン酸Na	964 μS/cm	964 μS/cm	1.03 mS/cm	986+/-38.1 μS/cm
5 mM コハク酸Na	1.05 mS/cm	960 μS/cm	1.01 mS/cm	1.01+/-0.045 mS/cm
5 mM シュウ酸Na	1.02 mS/cm	1.03 mS/cm	1.12 mS/cm	1.06+/-0.055 mS/cm
10 mM 酢酸Na	901 μS/cm	917 μS/cm	983 μS/cm	934+/-43.5 μS/cm
250 mM システイン	27.4 μS/cm	17.3 μS/cm	23.5 μS/cm	22.7+/-5.09 μS/cm
ミリーQ水	<0.5 μS/cm			0.1セルの検出限界より低い

表 1

The zwitter-ion buffer solution / conductivity / transportation rate pI or near [its] those, when the zwitter-ion buffer solution (good, the buffer solution, the amino acid buffer solution) or the tris-boric-acid buffer solution is used, electrophoresis transportation of DNA reaches comparatively and a certain profitableness exists about a rate. these -- 1 -- this buffer solution can be comparatively used in high concentration, and increases buffer capacity -- making -- 2 -- it is obtaining the profitableness of the electrophoresis transport number with those conductivity high about the analyte (DNA) which it is quite lower than the buffer solution of other types, and carries out 3 attentions by the same concentration.

Zwitter-ion buffer capacity in the isoelectric point (pI) The amino acid buffer solution has a buffer property in those pl(s). or [that given amino acid has the "highest buffer capacity" in the pI] -- or since it may not have, some they have buffer capacity -- I will come out. Buffer capacity decreases 10 times about the ** pH unit difference between pI and electric dissociation exponent, and the amino acid which has three sorts of ionizable radicals (a histidine, a cysteine, a lysine, glutamic acid, aspartic acid, etc.) has the buffer capacity higher than the amino acid which generally has only two sorts of dissociation at those pl(s). For example, as compared with the alanine or glycine which has comparatively low buffer capacity, all of histidine pI=7.47, lysine pI=9.74, and glutamic-acid pI=3.22 have comparatively good buffer capacity by those pI(s) in those pI(s) (refer to 79-page drawing 48 and 80-page drawing 49 in L.Lehniger, Biochemistry, the 2nd edition, Worth Publishers, New York, 1975; a detail [A.]). Although the histidine has been recommended as the buffer solution used by gel electrophoresis, hybridization does not have a limping gait crack in this system. Refer to U.S. Pat. No. 4,936,963. A cysteine is in a more nearly in-between location about buffer capacity. For pI of a cysteine, electric dissociation exponent of 5.02 and alpha carboxyl group is [electric dissociation exponent of 8.33 and alpha amino group of electric dissociation exponent of 1.71 and the sulfhydryl] 10.78. It is shown that the acid / base titration curve of a 250mM cysteine have the "buffer capacity" at abbreviation pH 5 with a cysteine better than 20mM sodium phosphate.

pH4 thru/or in 6, the buffer capacity of a cysteine is more nearly intentionally [than 20mM sodium phosphate] excellent in especially high pH. However, in these pH range, the conductivity of a 250mM cysteine solution is very low about 23microS/cm as compared with 20mM sodium phosphate which has the value of about 2.9 100 times larger mS/cm. Drawing 1 shows the conductivity measured value of the various buffer solutions for transportation.

The electrophoresis techniques of the shoes developed 20 years or more ago are "those pI(s)."

It is based on the capacity to separate the protein in the zwitter-ion buffer solution. These techniques are isoelectric focusing and iso octopus FORESHISU (Isotachophoresis).

And it is called isoelectric point fractionation (B. Chapter 3 of "Gel Electrophoresis of proteins:A Practical Approach" by D.Hames and D.Rickwood and Chapter 4, IRL Press 1981 reference). About these application, some the amino acid buffer solutions and the good buffer solutions were altogether used in those pI(s) (refer to [of said bibliography / the 168 page table 2]).

Low ionic strength and DNA transportation in the low conductivity buffer solution A series of fluorescence checker board trials were performed using 5580 chips and ByTr-RCA5 fluorescence probe which carried out the coat of the agarose 2.5%. The following systems: (1)250mM HEPES (low conductivity), (2) 10microM The sodium succinate, (3) 10microM Quick (6 seconds) checker board addressing has been attained to all the sodium citrates and (4) distilled water. The result of a sodium citrate is shown in drawing 1 . On the other hand, the low conductivity of some types or a low ionic strength solution has checker board addressing which is a little good special feature, and quick DNA transportation (6 thru/or DNA are recording of 12 seconds of 80-micrometer pad) was attained using all these systems. Furthermore, since DNA (itself is the poly anion) is an electrolyte which exists in the bulk solution which offers conductivity, the DNA addressing APEX chip in distilled water is possible for it. Drawing 1 shows the top view of the APEX chip which used the histidine.

relation between an electrophoresis transportation rate, and a cation / anion kind the fact that the mobility of electric charge analyte kinds (DNA, protein, etc.) is related to the ionic strength of an electrolyte -- in addition -- moreover, the mobility is greatly influenced with the cation in an electrolyte, and the property of an anion kind (refer to "Capillary Electrophoresis:Principles and Practice"). This special point is shown in said Biopolymer, Vol.2, pp.231-236, and 1964 reference about DNA transportation. 232-page drawing 1 of this reference shows change of the DNA mobility in the case of using the electrolytic solution which has a univalent anion (Li+>Na+>K+> TMA) which is different with the same ionic strength. Fundamentally, a different cation has an association constant which is different by the DNA phosphoric-acid radical, and/or can change the hydration field of the perimeter of a DNA molecule, and this can be led to change of a transportation rate.

This invention relates to discovery of the electric-field molecule biological equipment and this invention persons relevant to [in / especially / an APEX minute electronic chip and equipment] amelioration or the various parameters which optimum-ize, the electrolytic solution (buffer solution), and other conditions for a

DNA transportation rate, DNA-hybridization reaction effectiveness, and total hybridization singularity. This invention relates to discovery of this invention person that the zwitter-ion buffer solution containing the amino acid histidine especially prepared by the concentration of about 50 mM(s) of low conductivity offers optimal conditions about both quick electrophoresis DNA transportation and an efficient hybridization reaction, ten to 100 mM pI (isoelectric point 7.47 [about]) or near the at a detail. Especially this profitableness of the histidine buffer solution is important for APEX chip mold equipment.
These special equipments (micro by contrast [machining mold equipment]) have a limitation about the amount of the current which can be impressed, and an electrical potential difference. This limitation makes [both] it difficult to attain quick transportation and efficient hybridization using the same buffer-solution system. DNA transportation was performed in the low conductivity buffer solution (a cysteine or alanine) which still causes transportation with quick current/electrical potential difference restricted in these cases. Under these conditions, although DNA was accumulated by the trial part, it did not hybridize efficiently. Changing the solution into the high salt buffer solution (a >100mM sodium chloride or sodium phosphate) after transportation in these low conductivity buffer solutions, subsequently this caused efficient hybridization by the trial part.
Table 2 shows the result about a single string trial which associates the parameter of buffer capacity, pH, and conductivity, and the DNA are recording which used APEX chip equipment and hybridization sensibility (effectiveness).

溶液	緩衝能 pH 4-10		pIでのpH	導電率 (μS)	相対DNA輸送速度	SA-ビオチンT12感度	DNAのハイブリダイゼーション感度
β-アラニン	pK_1 - 3.6 pK_2 - 10.2	+	7.3	10.0	+++++ (最高速)	3×10^6	
タウリン	pK_1 - 1.5 pK_2 - 8.7	+/-	4.6	4.5	++++	> 7.5×10^{10}	
システイン	pK_1 - 1.7 pK_2 - 8.3 pK_3 - 10.8	+/-	5.2	25.0	++++	3×10^7	7.5×10^{10}
ヒスチジン	pK_1 - 1.8 pK_2 - 6.0 pK_3 - 9.0	+++	7.6	212.0 (172.0 高純度)	+++	3×10^5	3×10^4
リジン	pK_1 - 2.2 pK_2 - 8.9 pK_3 - 10.3	++	9.6	477.0	++	> 7.5×10^{10}	
$NaPO_4$	複合体	+	7.4 1/	1,400.0	+ (最低速)		

表 2

Table 2 shows the effectiveness of the various zwitter-ion amino acid buffer solutions [the beta-alanine, a taurine, a cysteine, a histidine, a lysine, and sodium phosphate (it is not the zwitter-ion buffer solution)] over the hybridization of the conveyed target DNA to the specific capture DNA to a detail by the trial part. Generally conductivity is correlated with transportation under the same place conditions about transportation. The beta-alanine, a taurine, and a cysteine show the outstanding transportation, and a histidine shows good transportation, and a lysine and NaPO4 show remarkable transportation. "Standard DNA" which has the Pori anionic phosphoric-acid frame which carried out the electric charge of the DNA-hybridization sensibility to negative is reported.
1/ In addition to 20mM NaPO4 doubled with pH7.4, and hybridization sensibility, Table 2 shows the sensibility about streptoavidin / biotin DNA probe capture compatibility.
Table 2 shows clearly the correlation of DNA transportation (are recording) and low conductivity (the beta-alanine, a taurine, a cysteine, histidine). A table shows the sensibility which was excellent about the

compatibility reaction of the streptoavidin / biotin probe which uses the beta-alanine, a cysteine, and a histidine. As reflected in the sensibility data of Table 2, a histidine offers greatly 4 or more ********s of hybridization effectiveness better than either of other buffer solutions like a cysteine or 20mM NaPO4. The improvement to a cysteine is at least 10 times, is 102 times more at a detail, and is at least 104 times most at a detail. It is that it is shown that Table 2 has very good DNA-hybridization sensibility (effectiveness) about the histidine buffer solution to the most important thing. Therefore, a histidine is the only thing which offers both good transportation, and good DNA / DNA-hybridization effectiveness among the zwitter-ion amino acid buffer solutions by which the current trial was carried out.
It is believed that the low conductivity of a histidine buffer-solution system caused quick transportation (are recording). It is related with why the histidine buffer solution causes comparatively effective DNA/DNA hybridization, and there is possible explanation of shoes. One advantage may be the buffer capacity which was excellent in the histidine. It is the buffer solution the histidine excelled [buffer solution] in the pI of 7.47 under acidity or basic conditions (A. refer to L.Lehninger, Biochemistry, the 2nd edition, worth Publishers, New York, and 1975 or 80-page drawing 49). DNA is accumulated for hybridization and an APEX chip generates an acid with the positive electrode with which a histidine may buffer these conditions efficiently. As for a more important thing, protonation of the imidazole group of a histidine starts the inversion to the dication kind of a molecule under these acid conditions (pH<5). This dication kind that has alpha-amino group which just carried out the electric charge, and the imidazole group which just carried out the electric charge is the case where it is applied that it may help to stabilize the DNA/DNA hybrid which promoted hybridization and was formed with the positive electrode of an APEX chip. When a cation, a dication, and the poly cation reduce the repulsive force of the phosphoric-acid frame which carried out the electric charge to negative [on double-stranded-DNA structure], helping stabilization of a DNA/DNA hybrid is known.
Moreover, it may be said that DNA/DNA/histidine may form the stabilization addition product of some types of other electrochemical products (hydrogen peroxide etc.) generated with a positive electrode. Although this example uses the histidine produced naturally, it can fully apply this invention to other nature or the synthetic compound which has good buffer capacity and low conductivity (or zwitter-ion property), and has the property of stabilizing DNA hybridization by charge stabilization or addition product formation.

Although said invention was partly indicated in the detail by the approach of illustration aiming at clarification and an understanding, and an example, probably, it will be easily clear to this contractor in the knowledge of the professor of this invention that its it can accomplish, without a certain kind of modification and qualification deviating from the pneuma or the range of the attached claim.

[Translation done.]

DRAWINGS

[Drawing 1]

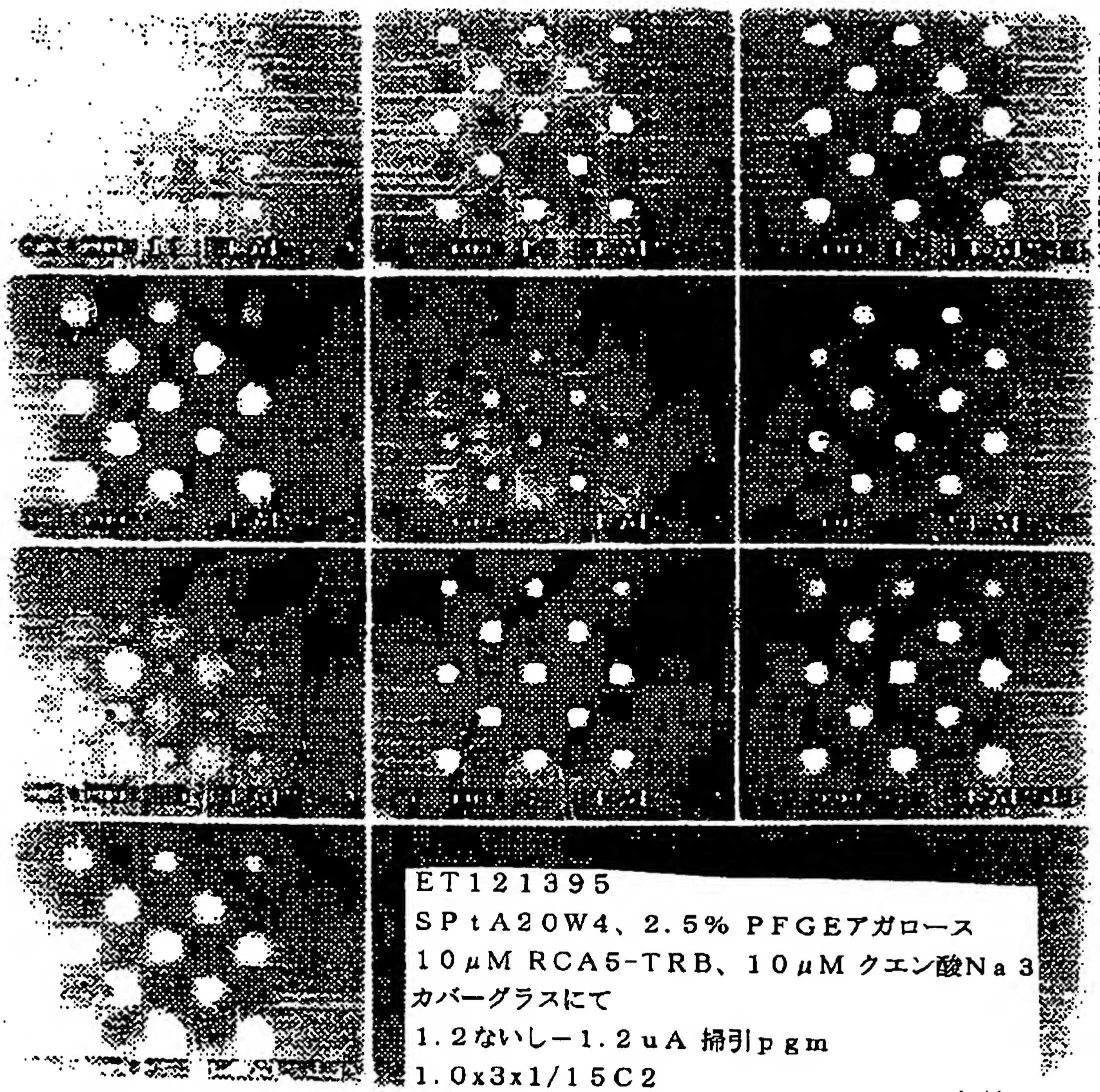

FIG. 1.

[Translation done.]

CORRECTION OR AMENDMENT

[Kind of official gazette] Printing of amendment by the convention of 2 of Article 17 of Patent Law
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C12N 15/09
C12Q  1/68
G01N 33/53
G01N 33/566
// C12M  1/00
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[FI]

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G01N 27/26    325 E
C12Q  1/68        A
G01N 33/53        M
G01N 33/566
C12N 15/00        A
C12M  1/00        A
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[Procedure revision]
[Filing Date] July 8, Heisei 16 (2004. 7.8)
[Procedure amendment 1]
[Document to be Amended] Specification
[Item(s) to be Amended] The passage of the contents of amendment
[Method of Amendment] Modification
[The contents of amendment]

手続補正書

特許庁長官殿

1．　事件の表示

平成１０年特許願第５１２６７６号

2．　補正をする者

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4．　補正により増加する請求項の数　１２

（別紙）

請　求　の　範　囲

1．超小型電子装置上の正に偏った試験部位での電場に

た一本鎖捕獲ＤＮＡに対するＤＮＡ分析物のハイブリタ

強する方法であって、

（１）該装置に緩衝液を適用し、ここに該緩衝液は、

（ａ）低導電率を有する緩衝液、

（ｂ）３．５を超える天然の等電点（ｐＩ）を有す

（ｃ）実質的にｐＨ４とｐＨ１０との間の有効な緩

により選択され、

（２）（ａ）超小型電子装置上の試験部位にて電場を

部位は、ＤＮＡ分析物に対して正に偏り、次いで

（ｂ）電場の影響下で緩衝分子に正味の正電荷を付

緩衝分子は、ＤＮＡ分析物と試験部位にて結合した一本

Ａハイブリダイゼーションを安定化させるように機能す

のに十分な量で超小型電子装置上の試験部位に電流を自

７．緩衝性存在物が、標的核酸および捕獲核酸間のハイ

化する請求項１記載の方法。

８．緩衝性存在物が低導電率を有する天然化合物である

９．緩衝性存在物が天然の、双性イオン化合物である請

１０．緩衝性存在物が低導電率を有する合成化合物であ

１１．緩衝性存在物が合成の、双性イオン化合物である

１２．ハイブリダイゼーション効率が、同一条件下のシ

なくとも１００倍大きい請求項１記載の方法。

１３．ハイブリダイゼーション効率が、同一条件下のシ

なくとも１，０００倍大きい請求項１記載の方法。

位置にて電場が生成されない第１の状態、および標的杉
れる第２の状態に少なくとも置かれるように印加する起
ション装置における標的核酸のハイブリダイゼーション
て、

緩衝液を該装置に適用し、

該装置に標的核酸を供し、

該装置へのパワーの適用を介して微小位置を該
の微小位置試験部位にて標的核酸の蓄積を引き起こし、

該第１の状態においてより、該第２の状態におい
捕獲核酸とをハイブリダイズさせる工程を含むことを特

１８．緩衝液が低導電率緩衝液である請求項１７記載の

１９．低導電率緩衝液が双性イオン緩衝液である請求項

２０．双性イオン緩衝液がヒスチジンを含む請求項１９

２１．ヒスチジンが約１０－１００ｍＭの濃度で調製され

２６．緩衝性存在物が天然の、双性イオン化合物であそ

２７．緩衝性存在物が低導電率を有する合成化合物であ

２８．緩衝性存在物が合成の、双性イオン化合物であそ

２９．ハイブリダイゼーション効率が、同一条件下のシ
なくとも１００倍大きい請求項１８記載の方法。

３０．ハイブリダイゼーション効率が、同一条件下のシ
なくとも１，０００倍大きい請求項１７記載の方法。

３１．ハイブリダイゼーション効率が、同一条件下のシ
なくとも約５０，０００倍大きい請求項１７記載の方法。

３２．緩衝性存在物が、該第２の状態において捕獲核酸
低下させる請求項１７記載の方法。

３８. 緩衝液が、実質的に７．４を超える天然のｐＩを有

３９．緩衝分子が、試験部位の周囲の溶液を緩衝するこ

位にて生成された水素からの分析物に対する保護を供す

４０．正味の正の荷電を有する緩衝分子がカチオンであ

４１．正味の正の荷電を有する緩衝分子がジカチオンて

４２. 正味の正の荷電を有する緩衝分子がポリカチオンで

４３．ＤＮＡのシールドを実質的に不可能とし、かくし

Ａハイブリダイゼーションを支持しないように、緩衝液

の方法。

４４．緩衝液が、捕獲核酸および標的核酸間の付加物形

記載の方法。

[Translation done.]